



We claim:

1. The present invention provides methods and software for tracking individual cells during a kinetic cell screening assay, comprising:

5 a) providing cells that possess at least a first luminescently labeled reporter molecule that reports on a cell structure;

b) obtaining a structure image from luminescent signals from the at least first luminescently labeled reporter molecule in the cells in a field of view;

c) creating a structure mask for individual cells in the field of view;

d) defining a reference point of each structure mask;

10 e) assigning an cell identification to each reference point in the field of view;

f) repeating steps (b) through (e) at a second time point;

g) correlating cell identification between the first time point and the second time point by calculating a distance between reference points in the field of view at the first time point and reference points in the field of view at the second time point; and

15 h) defining a cell identification match by identifying reference points in the field of view at the first time point and reference points in the field of view at the second time point that are closest together.

20 2. The method of claim 1, further comprising repeating steps (f) - (h) a desired number of times, wherein determining the distance between reference points is done by determining a distance between reference points in successive time points, and wherein defining the closest cell identification match is done by defining the closest cell identification match in successive time points.

25 3. The method of claim 1 wherein a cell identification match is rejected if the cells identified as a cell identification match are farther apart than a user-defined limit.

4. The method of claim 3 further comprising assigning a quality score to the cell identification match based on a distance determined for a second closest cell
30 identification match, and wherein a cell identification match is rejected if the quality score is below a user-defined threshold for a quality score.

5. The method of claim 4 wherein the quality score is calculated by a method comprising dividing the difference between the distance between reference points in the closest cell identification match from the distance between reference points in the second closest cell identification match by the distance between reference points in the second closest cell identification match.

6. The method of claim 1 further comprising determining a total sum of all distances or distances squared for all possible cell identification matches in successive time points, wherein a smallest total sum of all distances or distances squared is defined as a closest set of cell identification matches.

7. The method of claim 6 further comprising assigning a quality score to the cell identification match based on a sum of distances or distances squared determined for a second closest cell identification match, and wherein a cell identification match is rejected if the quality score is below a user-defined threshold for a quality score.

8. The method of claim 7 further comprising excluding from the determining a total sum of all distances or distances squared for all possible cell identification matches in successive time points any cell identification matches with a quality score at or above a user-defined threshold for quality scores.

9. The method of claim 8 wherein defining the cell identification match further comprises comparing other features of the individual cells between successive time points.

10. The method of claim 9 wherein the features comprise one or more features selected from the group consisting of area, shape, size, and luminescent intensity of cell or subcellular structures, and exogenous tags associated with cell or subcellular structures.

11. The method of claim 10 wherein the feature comprises an exogenous tag, and wherein the exogenous tag comprises a bar coding tag.
12. The method of claim 10 further comprising excluding any cell identification matches with a quality score at or above a user-defined threshold for quality scores from the comparing other characteristic features of the individual cells between successive time points.
13. The method of claim 10 further comprising excluding any cell identification matches with a quality score below a user-defined threshold for quality scores from the comparing other characteristic features of the individual cells between successive time points.
14. The method of claim 4 wherein the quality score is averaged over multiple time points and applied to a later time point as an average quality score.
15. The method of claim 7 further comprising determining a distance moved by the individual cell in successive time points.
16. The method of claim 15 further comprising determining an average distance moved by an individual cell over multiple time points.
17. The method of claim 1 wherein the cell structure is a nucleus, wherein the structure image is a nuclear image, and wherein the structure mask is a nuclear mask.
18. The method of claim 1, wherein the cell screening assay comprises one or more assays for the kinetic analysis of a cell parameter selected from the group consisting of ionic concentration, pH, gene expression, DNA proliferation, DNA content, cell viability, membrane potential, production of reactive oxygen species, enzyme activity, receptor activation, ligand binding, and transporter activity.

19. The method of claim 18, wherein the cells further possess at least a second luminescently labeled reporter molecule that reports on the cell parameter, and wherein the method further comprises obtaining luminescent signals from the second luminescently labeled reporter molecule and calculating a kinetic measure of the luminescent signals from the second luminescently labeled reporter molecule in individual cells, wherein the kinetic measure is selected from the group consisting of dynamic changes in intensity over time, heterogeneity of intensity among cells, oscillations in intensity, waves of intensity changes through connected cells, subpopulations of responding cells, and sequential activation of signaling molecules.

20. The method of claim 19, wherein the kinetic measure is modified, sorted, and/or excluded depending on a quality score for the cell identification match for each cell.

21. A computer readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute procedures for tracking individual cells during a kinetic cell screening assay, wherein the procedures comprise

a) providing cells that possess at least a first luminescently labeled reporter molecule that reports on a cell structure;

b) obtaining a structure image from luminescent signals from the at least first luminescently labeled reporter molecule in the cells in a field of view;

c) creating a structure mask for individual cells in the field of view;

d) defining a reference point of each structure mask;

e) assigning an cell identification to each reference point in the field of view;

f) repeating steps (b) through (e) at a second time point;

g) correlating cell identification between the first time point and the second time point by calculating a distance between reference points in the field of view at the first time point and reference points in the field of view at the second time point; and

h) defining a cell identification match by identifying reference points in the field of view at the first time point and reference points in the field of view at the second time point that are closest together.